

SUPPLEMENTARY INFORMATION

Title: Genome mining identifies cepacin as a plant-protective metabolite of the biopesticidal bacterium *Burkholderia ambifaria*

Short title: *Burkholderia ambifaria*: Genomics and biology of a biopesticide

Authors:

Alex J. Mullins¹, James. A. H. Murray¹, Matthew J. Bull¹, Matthew Jenner², Cerith Jones^{1,6}, Gordon Webster¹, Angharad E. Green³, Daniel R. Neill³, Thomas R. Connor¹, Julian Parkhill⁴, Gregory L. Challis^{2,5} and Eshwar Mahenthiralingam¹

Affiliations:

¹Microbiomes, Microbes and Informatics Group, Organisms and Environment Division, School of Biosciences, Cardiff University, Cardiff CF10 3AX, United Kingdom

²Department of Chemistry and Warwick Integrative Synthetic Biology Centre, University of Warwick, Coventry CV4 7AL, United Kingdom

³Institute of Infection and Global Health, University of Liverpool, Liverpool L69 7BE, United Kingdom

⁴Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom

⁵Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia

⁶Current address: Faculty of Computing, Engineering and Science, University of South Wales, Pontypridd, CF37 1DL, United Kingdom

Correspondence:

Submission and handling: Prof. Eshwar Mahenthiralingam, Microbiomes, Microbes and Informatics Group, Organisms and Environment Division, School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX, United Kingdom; Tel. +44 (0)29 20875875; Fax. +44 (0)29 20874305;

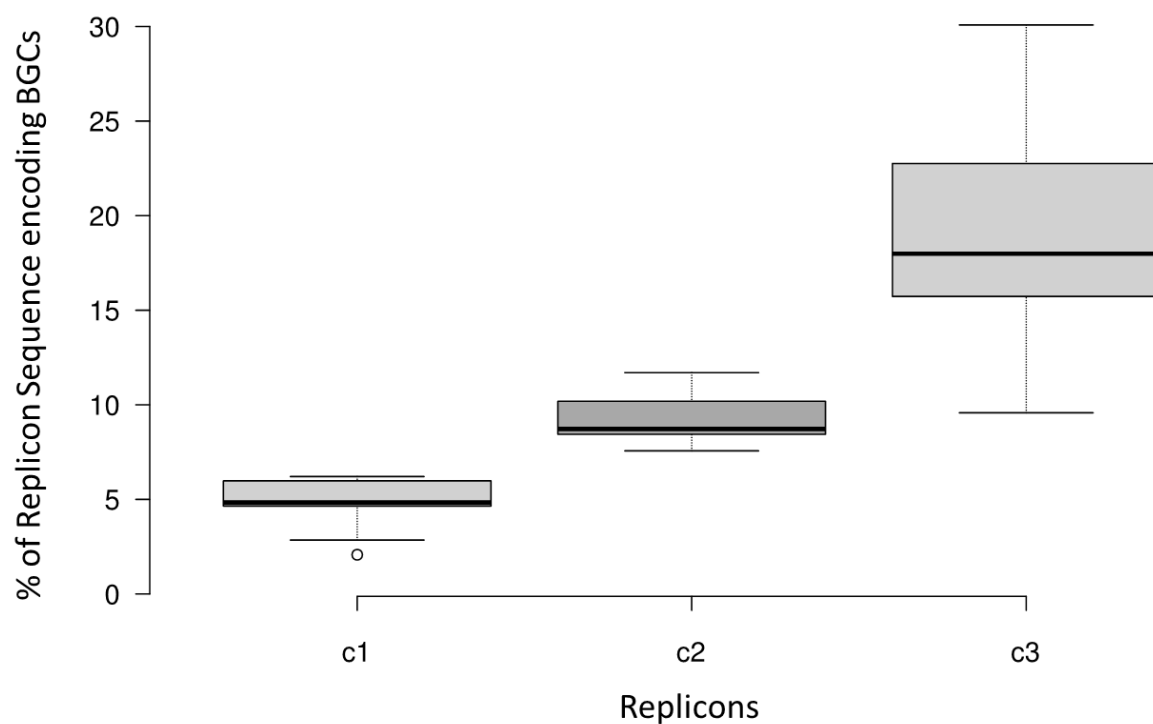
Email: MahenthiralingamE@cardiff.ac.uk (ORCID: 0000-0001-9014-3790)

Joint correspondence: Alex J. Mullins, Microbiomes, Microbes and Informatics Group, Organisms and Environment Division, School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX, United Kingdom; Tel. +44 (0)29 20874648

Email: MullinsA@cardiff.ac.uk (ORCID: 0000-0001-5804-9008)

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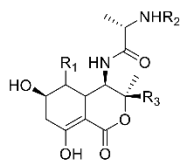
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Supplementary Figure 1. Distribution of secondary metabolite biosynthetic potential across the three replicons of 64 *B. ambifaria* strains. Centre lines represent the median; box limits indicate the 25th and 75th percentiles; and whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Only 63 strains are represented by the box plot of replicon c3 due to the lack of a third replicon in *B. ambifaria* BCC1105. Boxplots were generated using the web tool BoxPlotR⁵.

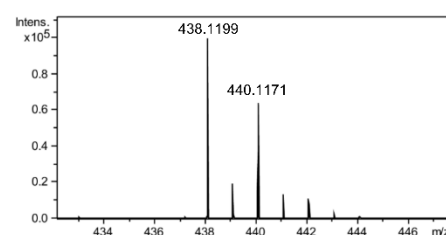
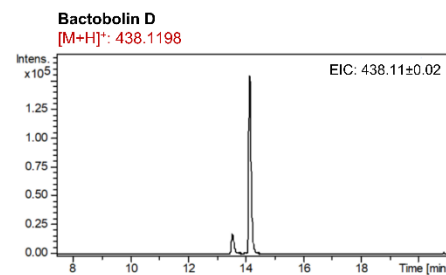
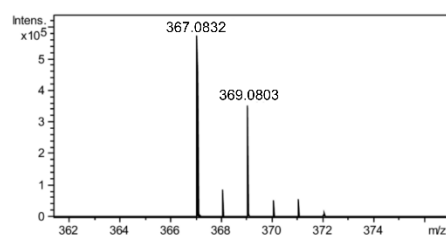
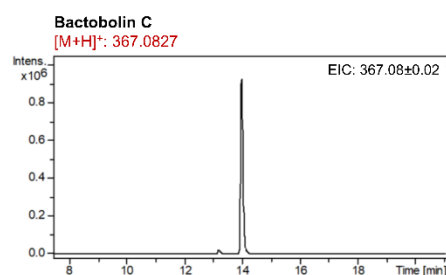
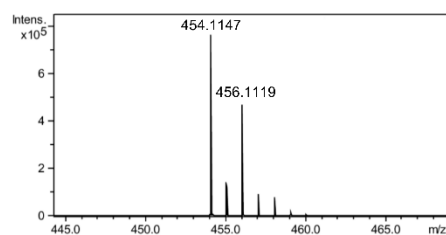
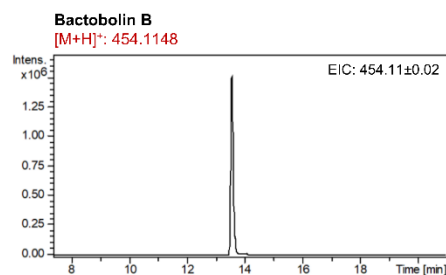
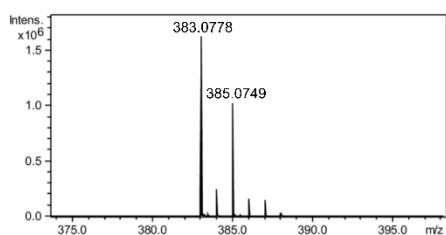
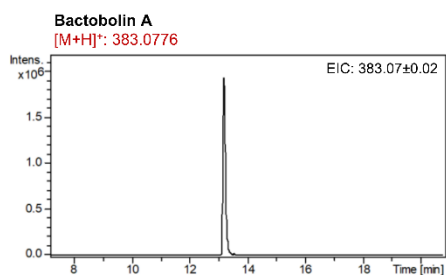
Supplementary Figure 2. LC-MS data for observed and theoretical mass(es), and mass spectrum for each antimicrobial metabolite detected in screened *B. ambifaria* strains. (a) Bactobolins; (b) Pyrrolnitrin; (c) Enacyloxin IIa; (d) Hydroxyquinolines; and (e) Burkholdines. To profile the metabolites produced by each strain under agar-growth condition (see Methods), $n = 1$ LC-MS analysis per strain (10 strains examined) (Table 1).

Supplementary Figure 2 (a) Bactobolins (A to D)

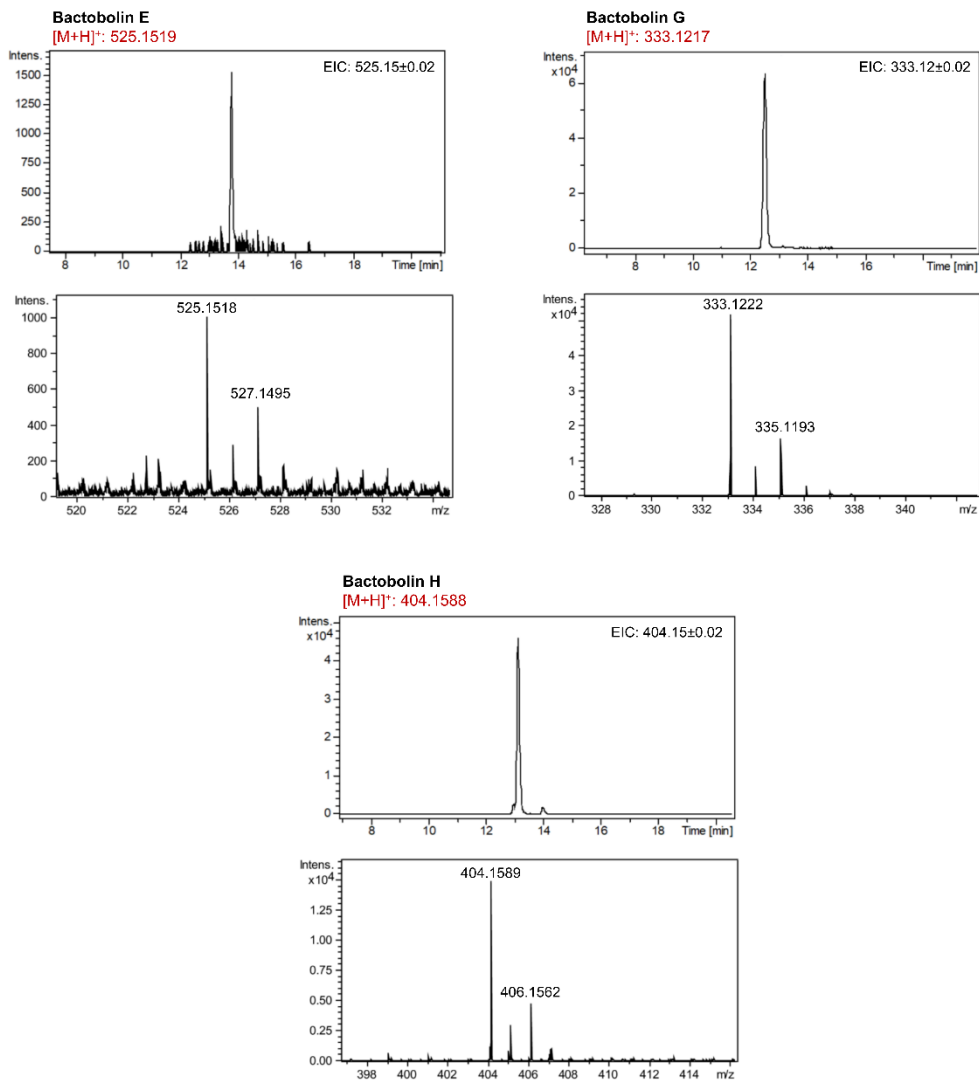


Bactobolins

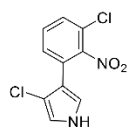
| | Chemical Formula | Exact Mass |
|---|--------------------------|------------|
| A: $R_1=OH, R_2=H, R_3=CHCl_2$ | $C_{14}H_{20}Cl_2N_2O_6$ | 382.0698 |
| B: $R_1=OH, R_2=L-Ala, R_3=CHCl_2$ | $C_{17}H_{26}Cl_2N_3O_7$ | 453.1070 |
| C: $R_1=H, R_2=H, R_3=CHCl_2$ | $C_{14}H_{20}Cl_2N_2O_5$ | 366.0749 |
| D: $R_1=H, R_2=L-Ala, R_3=CHCl_2$ | $C_{17}H_{26}Cl_2N_3O_6$ | 437.1120 |
| E: $R_1=OH, R_2=L-Ala-L-Ala, R_3=CHCl_2$ | $C_{20}H_{30}Cl_2N_4O_8$ | 524.1441 |
| G: $R_1=H, R_2=H, R_3=CH_2Cl$ | $C_{14}H_{21}ClN_2O_5$ | 332.1139 |
| H: $R_1=H, R_2=L-Ala, R_3=CH_2Cl$ | $C_{17}H_{26}ClN_3O_6$ | 403.1510 |



Supplementary Figure 2 (a) Bactobolins (E to H)



Supplementary Figure 2 (b) Pyrrolnitrin

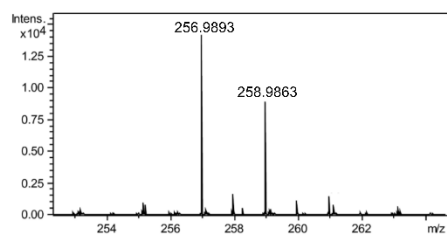
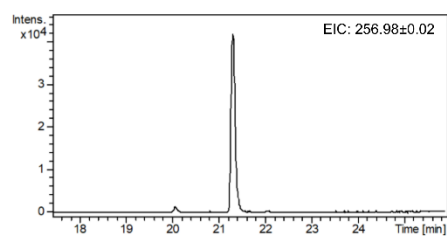


Pyrrolnitrin

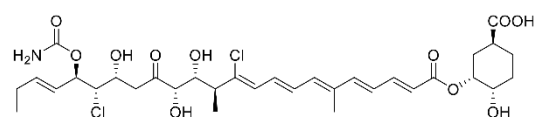
Chemical Formula: $C_{10}H_6Cl_2N_2O_2$

Exact Mass: 255.9806

$[M+H]^+$: 256.9884



Supplementary Figure 2 (c) Enacyloxin IIa

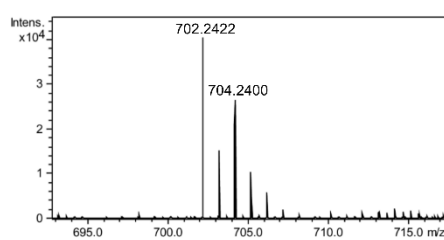
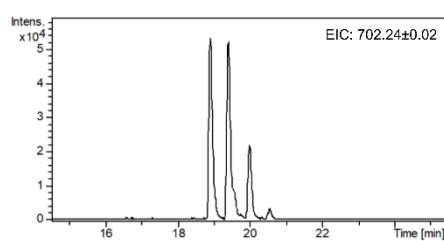


Enacyloxin IIa

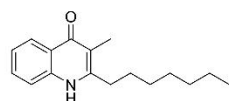
Chemical Formula: C₃₃H₄₆Cl₂NO₁₁

Exact Mass: 701.2370

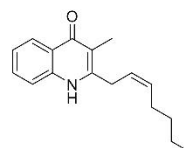
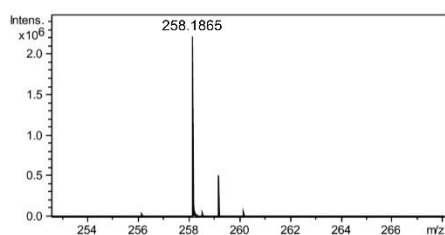
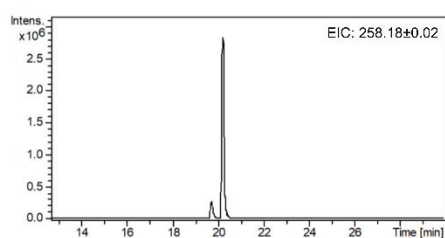
[M+H]⁺: 702.2447



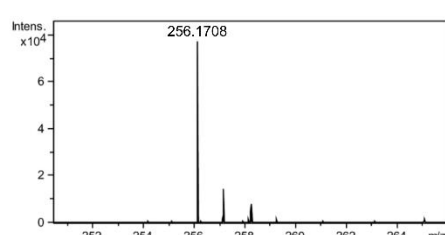
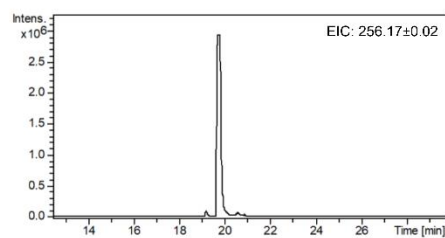
Supplementary Figure 2 (d) Hydroxyquinolines



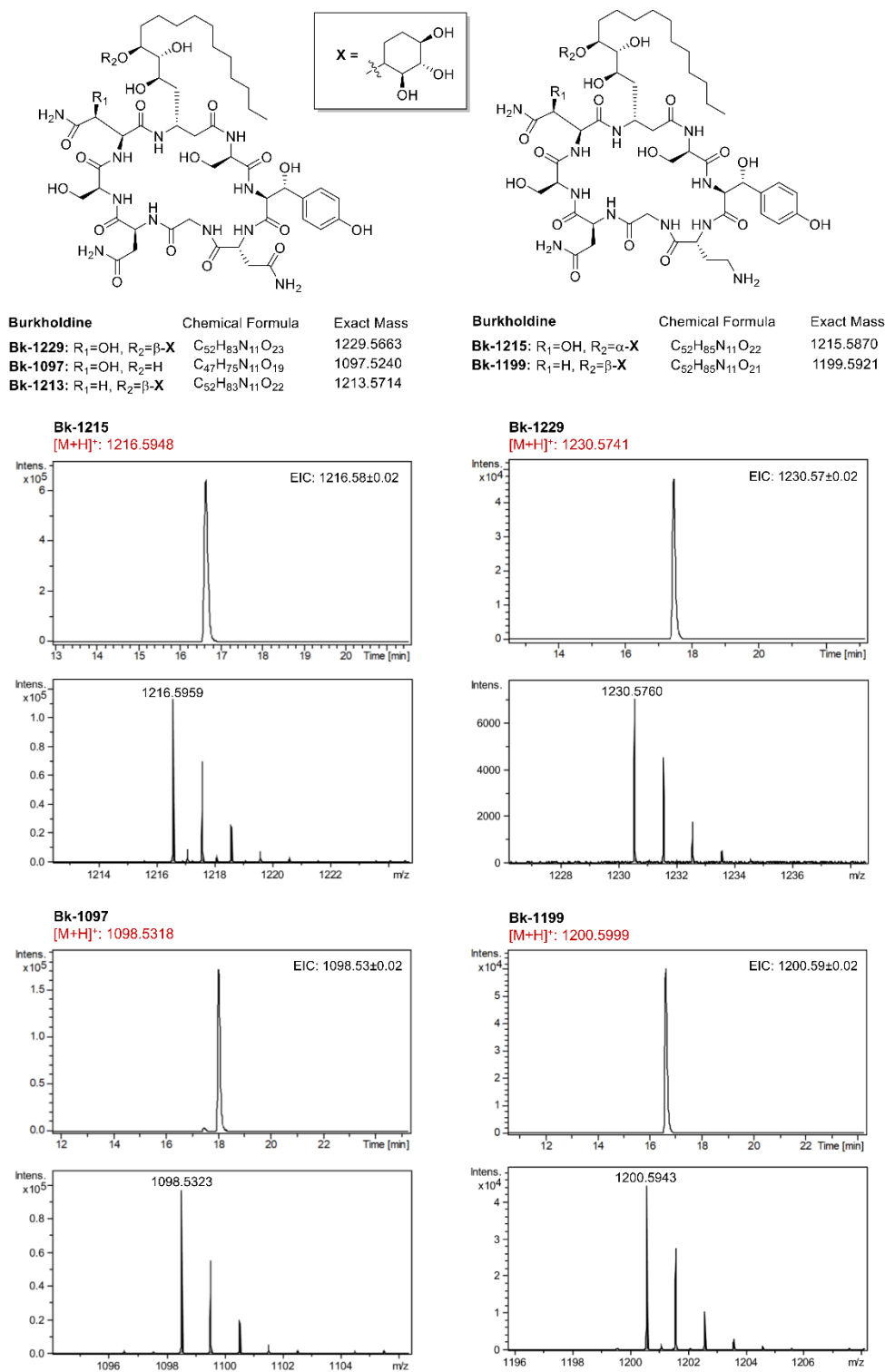
2-heptyl-3-methylquinolin-4(1H)-one
 Chemical Formula: $C_{17}H_{23}NO$
 Exact Mass: 257.1780
 $[M+H]^+$: 258.1858



(Z)-2-(hept-2-en-1-yl)-3-methylquinolin-4(1H)-one
 Chemical Formula: $C_{17}H_{21}NO$
 Exact Mass: 255.1623
 $[M+H]^+$: 256.1701

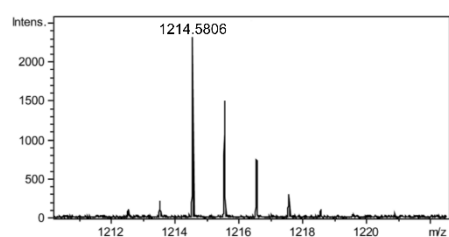
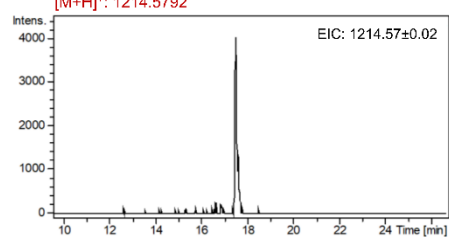


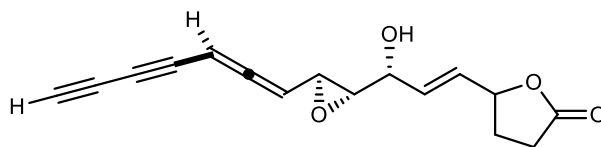
Supplementary Figure 2 (e) Burkholdines



Bk-1213

[M+H]⁺: 1214.5792





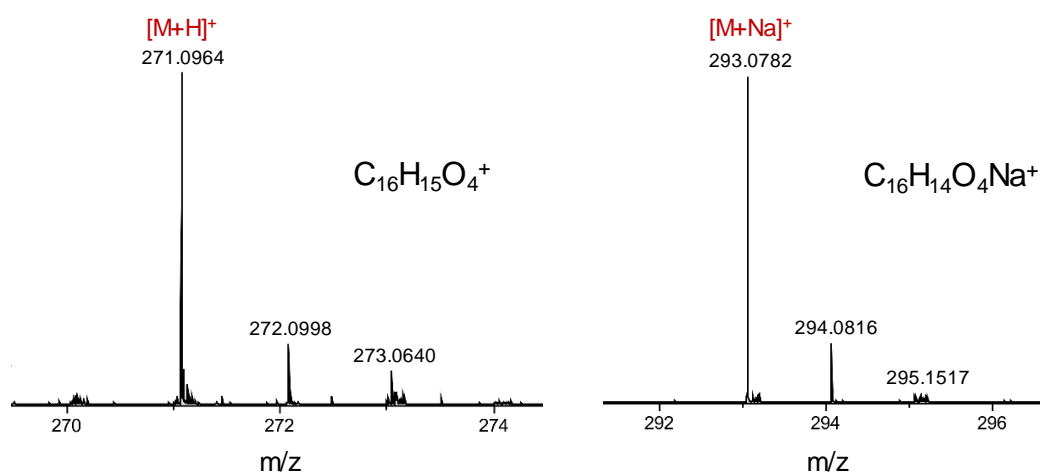
Cepacin A

Chemical Formula: $C_{16}H_{14}O_4$

Exact Mass: 270.0892

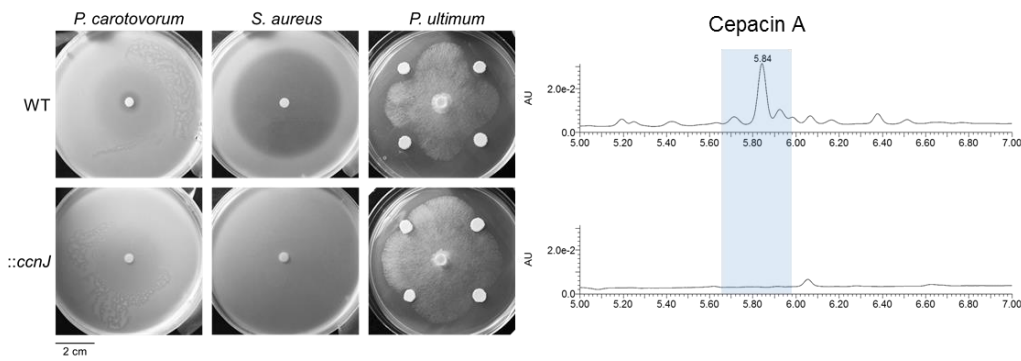
$[M+H]^+$: 271.0965

$[M+Na]^+$: 293.0784

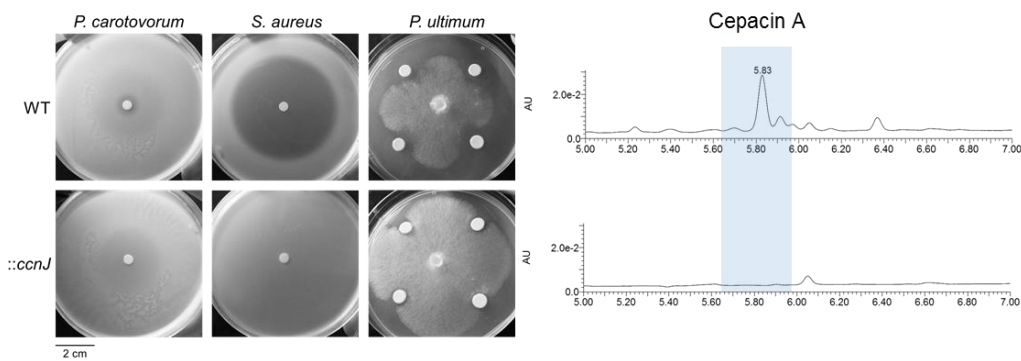


Supplementary Figure 3. High-resolution mass spectrometry analysis of *B. ambifaria* BCC0191 cepacin A. Measured spectra of cepacin A $[M+H]^+$ (left) and $[M+Na]^+$ (right). The generated molecular formulae for each species are shown and are in agreement with the molecular formula of cepacin A. $n = 3$ independent LC-MS analyses on agar grown cultures of strain BCC0191.

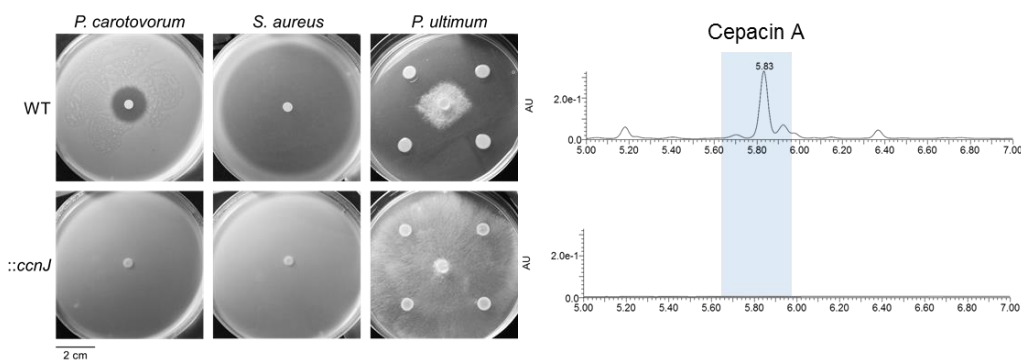
a) *B. ambifaria* BCC0191



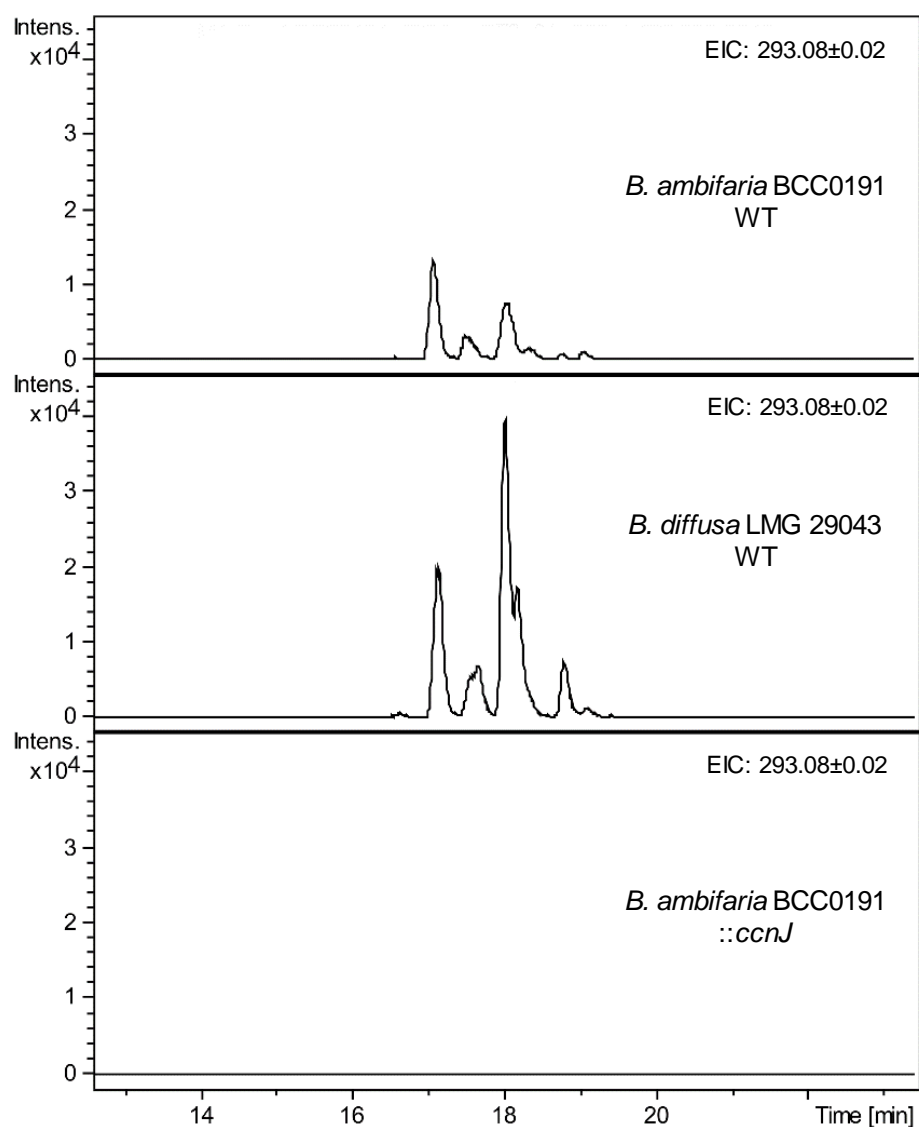
b) *B. ambifaria* BCC1252



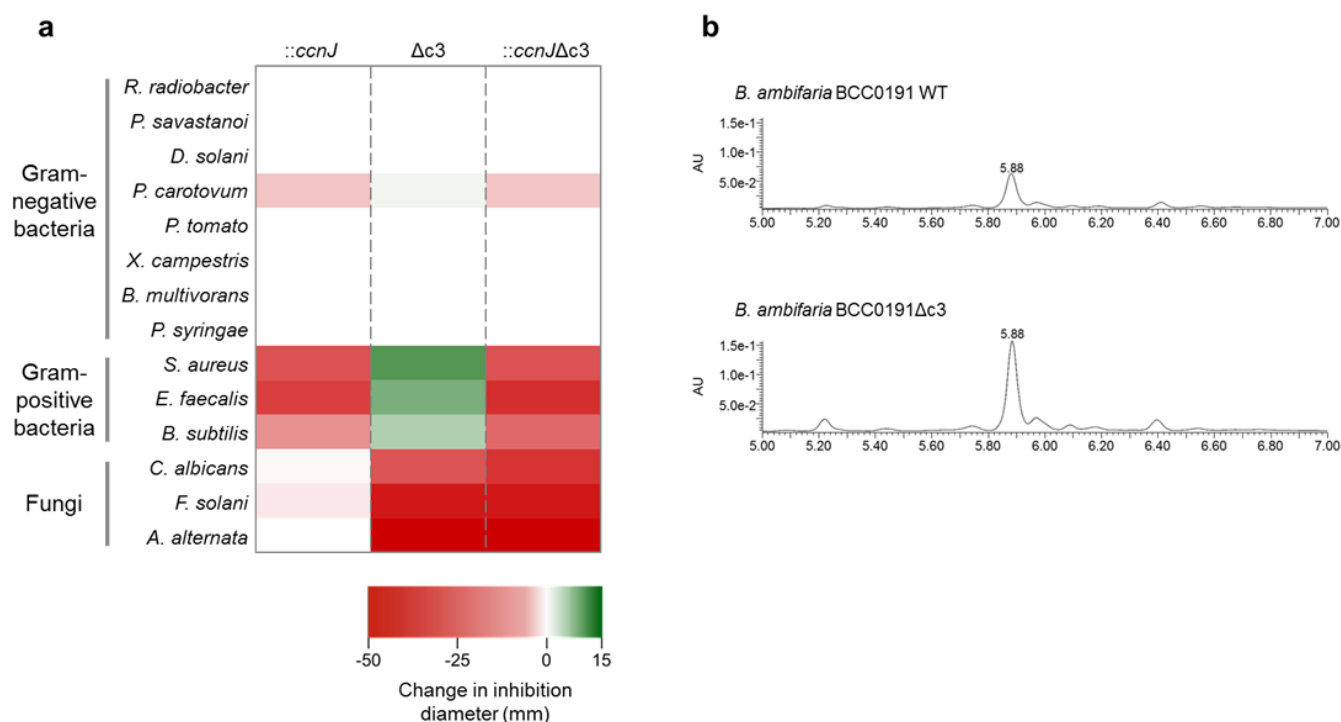
c) *B. ambifaria* BCC1241



Supplementary Figure 4. Antimicrobial activity, HPLC chromatograms and presence/absence of cepacin-related HPLC peaks for *B. ambifaria* wild-type (WT) and insertional mutants (*::ccnJ*). Antimicrobial activity against *P. carotovorum*, *S. aureus*, and *P. ultimum*; and HPLC chromatograms at 260 nm of the WT (see Supplementary Methods) and cepacin-deficient derivatives of *B. ambifaria* strains (a) BCC0191, (b) BCC1252, and (c) BCC1241 are shown. The absence of cepacin production in the cepacin-deficient derivatives of *B. ambifaria* strains BCC0477, BCC1259 and BCC1218 was also confirmed by HPLC analysis. $n = 2$ biological replicates per overlay; and $n = 2$ HPLC analyses for each strain wild type and mutant.

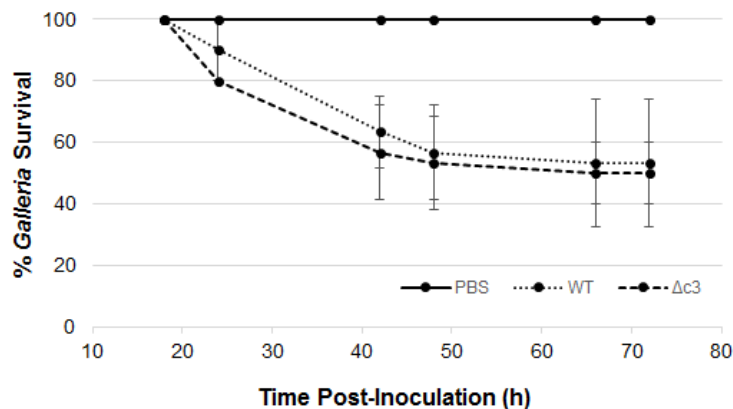


Supplementary Figure 5. Comparative cepacin A metabolite analysis of the original producer strain *B. diffusa* LMG 029043 and *B. ambifaria* BCC0191. Extracted ion chromatograms at m/z 293.08 \pm 0.02, corresponding to the $[M + Na]^+$ ion of cepacin A, from LC-MS analyses of crude extracts from agar-grown cultures of *B. ambifaria* BCC0191 (top), *B. diffusa* LMG 29043 (middle), and the insertional mutant *B. ambifaria* BCC0191::*ccnJ* (bottom). $n = 1$ independent LC-MS analysis per strain/derivative.

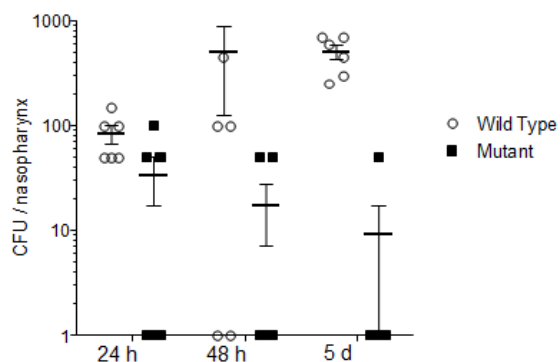


Supplementary Figure 6. Impact of mutations on *B. ambifaria* BCC0191 antimicrobial activity and cepacin production. (a) Three mutants were compared to BCC0191 wild-type (WT): cepacin-deficient derivative (::ccnJ), third replicon knockout ($\Delta c3$), and a combined mutation (::ccnJ $\Delta c3$). $n = 2$ independent overlays per condition, and the mean average used to generate the heat map. Scale represents change in zone of inhibition diameter (mm) compared to BCC0191 WT (red = reduced zone, white = no change, green = increased zone). (b) HPLC chromatograms at 260 nm of *B. ambifaria* BCC0191 WT and BCC0191 $\Delta c3$ ($n = 6$ independent HPLC analyses per strain) highlighting the impact of third replicon deletion on cepacin production.

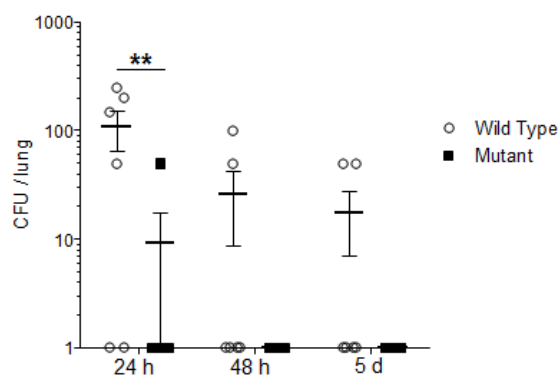
(a) Insect model



(b) Murine model - nasopharynx



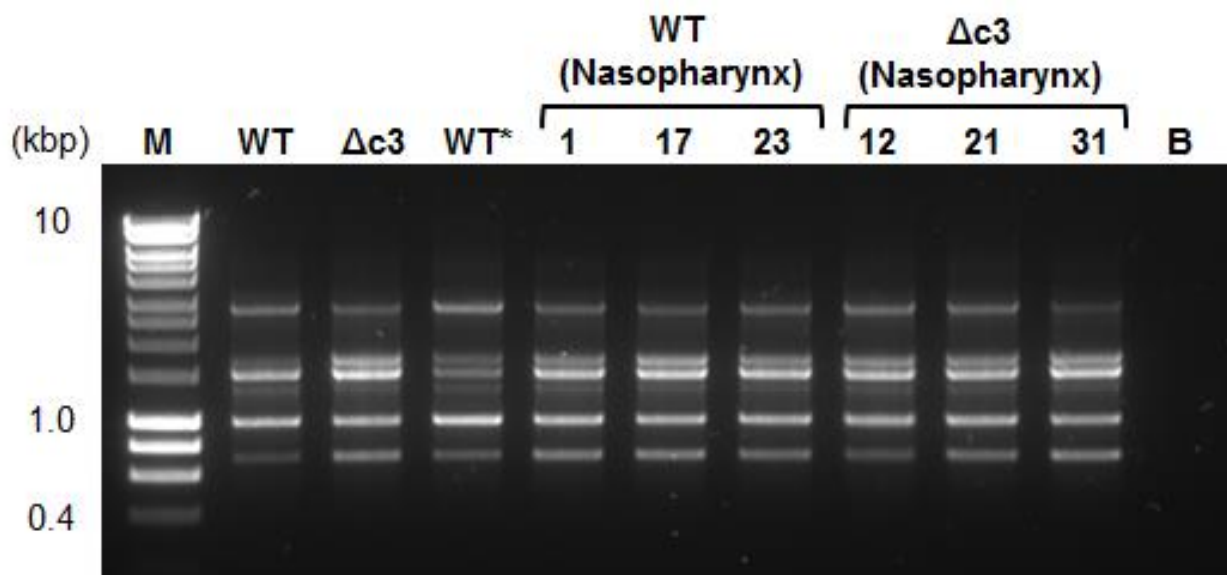
(c) Murine model - lung



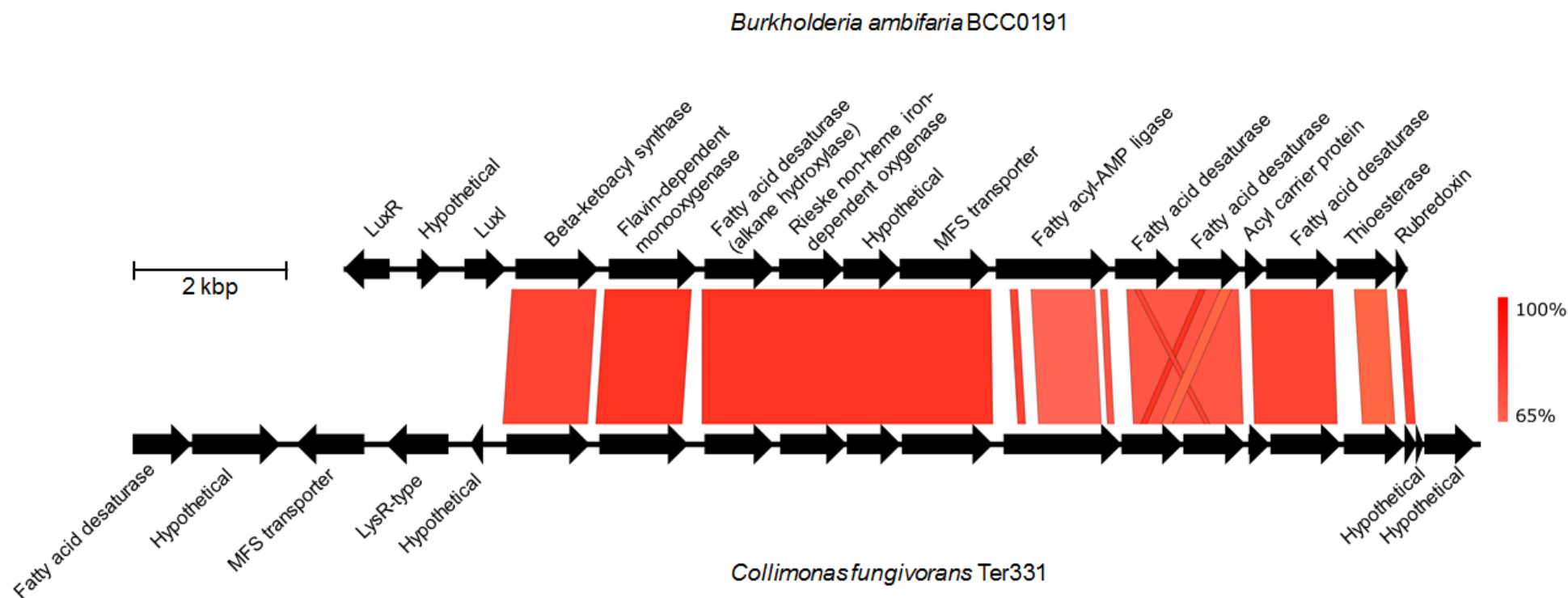
Supplementary Figure 7. Persistence of *B. ambifaria* BCC0191 and its third replicon mutant in models of infection.

***Galleria mellonella* wax moth larvae model (a).** Groups of 10 *Galleria mellonella* wax moth larvae, were injected in the last proleg on the right-side of the abdomen using a restraining technique as described⁶ ($n = 30$ larvae per condition). The larvae were incubated at 37°C for 72 hours, and their survival status was monitored periodically. Larvae were recorded as dead when they failed to respond to physical agitation. Centre bar represents the mean, and error bars represent standard deviation.

Murine model of respiratory tract infection: (b) nasopharynx and (c) lung. Mice ($n = 6$ mice per time point) were infected via inhalation with a dose of 2×10^6 *B. ambifaria* BCC0191 (wild type) and its corresponding third replicon deletion mutant, BCC0191 $\Delta c3$ (mutant), respectively. The mean number of colony forming units recovered from the nasopharynx (a) and lung (b) are shown after 24 hours, 48 hours and 5 days of infection. The centre bar represents the mean, and error bars represent the standard error. Persistence of the third replicon mutant after 24 hours in the lung was significantly lower than the wild type (** = $P = 0.0038$ in two-way ANOVA with Sidak's multiple comparisons test of wild type vs mutant). No colony forming units of the third replicon mutant were detected in the lungs of mice after 48 hours and 5 days of infection.



Supplementary Figure 8. PCR genotyping of *B. ambifaria* BCC0191 and BCC0191 Δ c3 recovered from the murine respiratory infection model. DNA was extracted from bacteria recovered from the murine infection model ($n = 31$ samples) using chelex-100 resin and random amplified polymorphic DNA (RAPD) fingerprinting PCR⁷ was performed ($n = 1$ independent PCR per sample) to validate their genetic identity. PCR products separated by agarose gel electrophoresis, and lanes are as follows: WT, strain BCC0191; Δ c3, BCC0191 Δ c3; WT*, BCC0191 high quality DNA extracted using Maxwell16 instrument; 1, 17 and 23, BCC0191 WT colonies recovered from the nasopharynx of mice at 24 hours, 48 hours and 5 days of infection, respectively; 12, 21, and 31, BCC0191:: Δ c3 mutant colonies recovered from the nasopharynx of mice at 24 hours, 48 hours and 5 days of infection, respectively; and B, PCR blank with no template DNA. The gel is representative of RAPD PCR genotyping performed on the 31 bacterial samples archived post infection from the nasopharynx or lung (24 BCC0191 WT and 7 BCC0191 Δ c3), and all colonies produced identical RAPD PCR profiles.



Supplementary Figure 9. Comparison of the gene organization for the *Burkholderia* cepacin A and *Collimonas* collimomycin BGCs. The predicted core biosynthesis genes are highly conserved between the pathways. The *Collimonas* BGC possesses several additional genes, mostly of hypothetical function, and the regulatory component upstream of the core biosynthetic genes differs between the two species. The sequence comparison image was constructed using the Python application Easyfig⁸.

Supplementary Table 1. *B. ambifaria* strains and genomes used during this study.

| Study Strain Name | Alternative Name | Source details | Accession No. or Bioproject | Reference |
|----------------------|--|--|-----------------------------|------------|
| BCC0118 | CEP0617; LMG P-24636 | CF (Sputum); USA | ERS784989 | / |
| BCC0191 | HI 2345 (J82); ATCC 51993; ARS BcB | ENV (Soil); USA | ERS784799 | 11 |
| BCC0192 | Ral-3; R-8863; HI2347; FC627 | ENV (Corn rhizosphere, biocontrol strain); USA | ERS785047 | 12 |
| BCC0197 | ATCC 51671; LMG 19465; FC661; R-9945; B37w | ENV (Leaves of <i>Sesbania exaltata</i> , biocontrol strain) | ERS785076 | 13 |
| BCC0200 | Formally <i>B. cepacia</i> gv I (BC-B) | ENV; USA | ERS785045 | 11 |
| BCC0203 | BCF/HG1-A; LMG-P 24640 | ENV; USA | ERS782625 | 14 |
| BCC0207 | AMMD (LMG 19182 ^T) | ENV (Pea rhizosphere); USA | PRJNA13490 | 12 |
| BCC0250 | CEP0958; LMG P-24637; R-9927 | CF (Sputum); Australia | ERS784819 | / |
| BCC0267 | LMG 19467; CEP0996; R-9935 | CF (Sputum); Australia | ERS784835 | 12 |
| BCC0284 | ATCC 53267; LMG 17829; CEP0102; C2965 | ENV (Corn roots); USA | ERS1328916 | 12 |
| BCC0316 | M54, HI 2347, R-5142 | ENV (Soil); USA | ERS784850 | 12 |
| BCC0338 | ATCC 53266 LMG 17828; FC662 | ENV (Corn roots); USA | ERS1371637 | 12 |
| BCC0399 | CEP1054 | CF; USA | ERS784860 | / |
| BCC0410 | MVP/C1 64 | ENV (Maize); Italy | ERS784866 | 15 |
| BCC0423 | MCI 4 | ENV (Maize); Italy | ERS1336067 | 16 |
| BCC0477 | AU0216 | CF (Sputum); USA | ERS784882 | / |
| BCC0478 | AU1366 | CF (Sputum); USA | ERS784897 | / |
| BCC0480 | HI-2427 | ENV (Soil); USA | ERS784913 | / |
| BCC1041 | MVP-C2-51 | ENV (Maize); Italy | ERS784930 | 15 |
| BCC1048 | MVP-C2-69 | ENV (Maize); Italy | ERS784886 | 15 |
| BCC1052 | MCII-68 | ENV (Maize); Italy | ERS1371632 | 17 |
| BCC1062 | MDII-130riz | ENV (Maize); Italy | ERS1328829 | 18 |
| BCC1065 | MDIII-B-388 | ENV (Maize); Italy | ERS784959 | 18 |
| BCC1066 | MDIII-B-399 | ENV (Maize); Italy | ERS784800 | 18 |
| BCC1072 | MDIII-P-170 | ENV (Maize); Italy | ERS1328913 | 18 |
| BCC1080 | MDIII-T-2 | ENV (Maize); Italy | ERS1371635 | 18 |
| BCC1083 | MDIII-T-50 | ENV (Maize); Italy | ERS1328835 | 18 |
| BCC1086 | MDIII-T-401(s) | ENV (Maize); Italy | ERS1328827 | 18 |
| BCC1088 | MDIII-T-474(s) | ENV (Maize); Italy | ERS1371633 | 18 |
| BCC1090 | MVP-C1-40 | ENV (Maize); Italy | ERS1328833 | 15 |
| BCC1092 | MVP-C1-53 | ENV (Maize); Italy | ERS784808 | 15 |
| BCC1093 | MVP-C1-55 | ENV (Maize); Italy | ERS784821 | 15 |
| BCC1095 | MVP-C1-80 | ENV (Maize); Italy | ERS784837 | 15 |
| BCC1098 | MVP-C1-95 | ENV (Maize); Italy | ERS784852 | 15 |
| BCC1100 | MVP-C2-25 | ENV (Maize); Italy | ERS784868 | 15 |
| BCC1103 | MVP-C2-44 | ENV (Maize); Italy | ERS784884 | 15 |
| BCC1105 | MVP-C2-73 | ENV (Maize); Italy | ERS1371636 | 15 |
| BCC1107 | MVP-C2-79 | ENV (Maize); Italy | ERS784899 | 15 |
| BCC1212 | MC40-6 | ENV (Rhizosphere); USA | PRJNA17411 | / |
| BCC1213 | MC80-27 | ENV; USA | ERS1328957 | / |
| BCC1214 | MA80-5 | ENV; USA | ERS784915 | / |
| BCC1216 | MW20-13 | ENV; USA | ERS1328918 | / |
| BCC1218 | MW80-16 | ENV; USA | ERS784932 | / |
| BCC1220 | MS5-3 | ENV; USA | ERS1328837 | / |
| BCC1223 | MS80-4 | ENV; USA | ERS784947 | / |
| BCC1224 | KS0-1 | ENV (Maize); USA | ERS1328836 | 19 |
| BCC1228 | KA20-1 | ENV (Maize); USA | ERS1328832 | 19 |
| BCC1229 | KA5-1 | ENV (Maize); USA | ERS1371639 | 19 |
| BCC1233 | KC0-24 | ENV (Maize); USA | ERS1328917 | 19 |
| BCC1236 | KC5-54 | ENV (Maize); USA | ERS1328839 | 19 |
| BCC1237 | KC10-16 | ENV (Maize); USA | ERS1371640 | 19 |
| BCC1240 | KC311-11 | ENV (Maize); USA | ERS1328914 | 19 |
| BCC1241 | KC311-6 | ENV (Maize); USA | ERS784961 | 19 |
| BCC1246 | KC20-40 | ENV (Maize); USA | ERS1328834 | 19 |
| BCC1248 | KW0-1; LMG-P 24641 | ENV (Maize); USA | ERS784801 | 19 |
| BCC1249 | KW0-5 | ENV (Maize); USA | ERS1371634 | 19 |
| BCC1252 | KW10-1 | ENV (Maize); USA | ERS784809 | 19 |
| BCC1256 | KW420-19 | ENV (Maize); USA | ERS784823 | 19 |
| BCC1258 | KW318-1 | ENV (Maize); USA | ERS1371641 | 19 |
| BCC1259 | KW20-2 | ENV (Maize); USA | ERS784838 | 19 |
| BCC1265 | MC40-7 | ENV; USA | ERS784854 | / |
| BCC1270 | KC20-17 | ENV (Maize); USA | ERS784870 | 19 |
| IOP40-10 | / | ENV (Prairie grass rhizosphere) | PRJNA20669 | / |
| MEX-5 | / | ENV (Teosinte plants (<i>Zea perennis</i>)) | PRJNA20667 | / |
| BCC0191 ::ccnJ | / | / | / | This study |
| BCC1252 ::ccnJ | / | / | / | This study |
| BCC1241 ::ccnJ | / | / | / | This study |
| BCC0477 ::ccnJ | / | / | / | This study |
| BCC1259 ::ccnJ | / | / | / | This study |
| BCC1218 ::ccnJ | / | / | / | This study |
| BCC0191Δc3 | / | / | / | This study |
| BCC0191 ::ccnJΔc3 | / | / | / | This study |

Supplementary Table 2. *B. ambifaria* genomic statistics.

| Strain | Total Contigs (Mbp) | Mapped Contigs (Mbp) | Total Contig No. | Contigs <1000 bp | N50 (bp) |
|----------|---------------------|----------------------|------------------|------------------|----------|
| BCC0118 | 7.50 | 7.43 | 103 | 45 | 273879 |
| BCC0191 | 7.58 | 7.55 | 97 | 57 | 302202 |
| BCC0192 | 7.40 | 7.32 | 100 | 48 | 277504 |
| BCC0197 | 7.38 | 7.36 | 82 | 33 | 386867 |
| BCC0200 | 7.63 | 7.55 | 124 | 64 | 292321 |
| BCC0203 | 7.93 | 7.53 | 4 | 4 | 2669373 |
| BCC0207 | 7.53 | 7.48 | 4 | 4 | 2646969 |
| BCC0250 | 7.36 | 7.33 | 88 | 32 | 396147 |
| BCC0267 | 7.36 | 7.33 | 74 | 27 | 784052 |
| BCC0284 | 7.47 | 7.38 | 88 | 34 | 423851 |
| BCC0316 | 7.64 | 7.56 | 131 | 62 | 292321 |
| BCC0338 | 7.45 | 7.42 | 61 | 28 | 514723 |
| BCC0399 | 7.40 | 7.38 | 86 | 38 | 407605 |
| BCC0410 | 7.38 | 7.36 | 44 | 18 | 849830 |
| BCC0423 | 7.47 | 7.30 | 100 | 36 | 382759 |
| BCC0477 | 7.81 | 7.43 | 118 | 54 | 255598 |
| BCC0478 | 7.24 | 7.21 | 89 | 25 | 601161 |
| BCC0480 | 7.84 | 7.52 | 87 | 24 | 570779 |
| BCC1041 | 7.51 | 7.42 | 88 | 38 | 469567 |
| BCC1048 | 7.51 | 7.43 | 76 | 35 | 469249 |
| BCC1052 | 7.33 | 7.30 | 77 | 34 | 397259 |
| BCC1062 | 7.45 | 7.36 | 90 | 37 | 382455 |
| BCC1065 | 7.31 | 7.28 | 81 | 32 | 605534 |
| BCC1066 | 7.32 | 7.29 | 112 | 37 | 381533 |
| BCC1072 | 7.44 | 7.36 | 93 | 40 | 397405 |
| BCC1080 | 7.29 | 7.26 | 85 | 35 | 383339 |
| BCC1083 | 7.30 | 7.26 | 95 | 35 | 373328 |
| BCC1086 | 7.60 | 7.40 | 302 | 49 | 395516 |
| BCC1088 | 7.48 | 7.40 | 70 | 38 | 397405 |
| BCC1090 | 7.33 | 7.29 | 114 | 33 | 340920 |
| BCC1092 | 7.38 | 7.36 | 57 | 20 | 849686 |
| BCC1093 | 7.40 | 7.38 | 91 | 37 | 427669 |
| BCC1095 | 7.40 | 7.38 | 79 | 34 | 407577 |
| BCC1098 | 7.38 | 7.37 | 48 | 15 | 849506 |
| BCC1100 | 7.40 | 7.37 | 91 | 37 | 407581 |
| BCC1103 | 7.40 | 7.37 | 77 | 38 | 427387 |
| BCC1105 | 6.30 | 6.13 | 50 | 15 | 1592784 |
| BCC1107 | 7.40 | 7.37 | 83 | 39 | 374059 |
| BCC1213 | 7.47 | 7.42 | 107 | 40 | 425516 |
| BCC1214 | 7.61 | 7.14 | 169 | 79 | 270842 |
| BCC1216 | 7.46 | 7.33 | 166 | 73 | 234327 |
| BCC1218 | 7.46 | 7.42 | 109 | 51 | 348377 |
| BCC1220 | 7.64 | 7.14 | 188 | 74 | 393412 |
| BCC1223 | 7.61 | 7.14 | 157 | 80 | 267383 |
| BCC1224 | 7.27 | 7.19 | 152 | 53 | 360040 |
| BCC1228 | 7.38 | 7.35 | 82 | 33 | 558864 |
| BCC1229 | 7.51 | 7.48 | 100 | 51 | 298531 |
| BCC1233 | 7.96 | 7.42 | 126 | 75 | 390415 |
| BCC1236 | 7.63 | 7.58 | 123 | 47 | 358767 |
| BCC1237 | 7.34 | 7.31 | 91 | 32 | 420767 |
| BCC1240 | 7.40 | 7.37 | 63 | 29 | 503900 |
| BCC1241 | 7.68 | 7.44 | 138 | 61 | 288780 |
| BCC1246 | 7.48 | 7.44 | 72 | 32 | 450462 |
| BCC1248 | 8.03 | 7.50 | 273 | 132 | 241997 |
| BCC1249 | 7.58 | 7.55 | 71 | 27 | 447443 |
| BCC1252 | 7.42 | 7.38 | 107 | 60 | 254705 |
| BCC1256 | 7.54 | 7.47 | 104 | 49 | 281063 |
| BCC1258 | 7.60 | 7.52 | 110 | 57 | 272850 |
| BCC1259 | 7.63 | 7.30 | 162 | 75 | 431727 |
| BCC1265 | 7.62 | 7.29 | 136 | 50 | 335885 |
| BCC1270 | 7.60 | 7.53 | 115 | 51 | 315858 |
| IOP40-10 | 7.69 | 6.88 | 629 | 571 | 24355 |
| MC40-6 | 7.64 | 7.34 | 4 | 4 | 2769414 |
| MEX-5 | 7.86 | 6.58 | 706 | 634 | 20649 |

Supplementary Table 3. De-replicated secondary metabolite gene clusters of *B. ambifaria*.

| Cluster Type (antiSMASH prediction) | Prevalence in <i>B. ambifaria</i> (out of 64 genomes) | Average length (kbp) ^{a,b} |
|---|--|-------------------------------------|
| Replicon c1 | | |
| Terpene 1 | 65 ^c | 20.9 |
| NRPS 1 | 64 | 54.5 |
| Arylpolyene 1 | 64 | 41.2 |
| NRPS 2 | 44 | 46.8 |
| PKS 1 | 28 | 47.6 |
| Lantipeptide 1 | 10 | 27.1 |
| Butyrolactone 1 | 1 | 11.0 |
| Terpene 2 | 1 | 21.0 |
| Replicon c2 | | |
| Homoserine lactone 1 | 64 | 20.6 |
| Phosphonate | 64 | 41.7 |
| Terpene 3 | 64 | 21.1 |
| Other 1 | 64 | 41.1 |
| Terpene 4 | 64 | 24.0 |
| Bacteriocin 1 | 55 | 13.1 |
| Arylpolyene 2 | 53 | 44.9 |
| Ectoine 1 | 53 | 10.4 |
| Ectoine 2 | 38 | 10.4 |
| Homoserine lactone (cepacin-associated) 2 | 22 | 20.7 |
| Other 2 | 20 | 43.0 |
| Butyrolactone-otherKS | 16 | 32.5 |
| Phenazine | 13 | 20.4 |
| PKS 2 | 7 | 44.9 |
| NRPS 3 | 2 | 52.8 |
| Bacteriocin 2 | 1 | 25.9 |
| Arylpolyene 3 | 1 | 41.1 |
| Replicon c3 | | |
| Homoserine lactone 3 | 63 | 20.6 |
| Bacteriocin 3 | 63 | 10.8 |
| Terpene 5 | 63 | 22.0 |
| NRPS-T1PKS 1 | 54 ^d | 85.5 or 117.6 ^e |
| PKS 3 | 40 | 43.9 and 63.9 ^f |
| Other 3 | 34 | 27.0 |
| Butyrolactone 2 | 27 | 10.7 |
| Other 4 | 24 | 43.8 |
| NRPS-T1PKS 2 | 17 | 60.9 |
| <i>Trans</i> -AT-PKS | 6 | 91.2 |
| Unknown genomic location | | |
| NRPS 4 | 1 | 26.8 |
| Homoserine lactone 4 | 1 | 20.1 |
| NRPS-T1PKS- <i>trans</i> -AT-PKS | 1 | 69.8 |

Footnotes:

^a *B. ambifaria* strains MEX-5 and IOP40-10 were excluded from several cluster length averages due to the fragmented nature of clusters caused by lower quality genome assemblies. Other strains were excluded if the clusters were manually split from the antiSMASH predicted cluster.

^b Average length was calculated from predicted antiSMASH clusters or extracted sequences identified using BLAST; partial clusters were excluded.

^c *B. ambifaria* strain BCC1249 encoded a duplicate terpene 1 cluster.

^d *B. ambifaria* strains MEX-5, IOP40-10 and BCC1065 either possess low quality genomes preventing the reconstruction of the burkholdine NRPS-T1PKS pathway or encode a partial cluster.

^e The published NRPS-T1PKS pathway (85.5 kbp) was encoded by 36 *B. ambifaria* strains, while the larger 117.6 kbp pathway was encoded by 15 *B. ambifaria* strains and consists of the published pathway with additional biosynthetic genes.

^f The 43.9 kbp version of the pathway was encoded by 26 *B. ambifaria* strains, while the 63.9 kbp version was encoded by 14 *B. ambifaria* strains.

Supplementary Table 4. Known antimicrobial compounds produced by *B. ambifaria*.

| Compound | Prevalence in <i>B. ambifaria</i> | Replicon | Bioactivity activity |
|------------------------|-----------------------------------|----------|---------------------------------------|
| Pyrrolnitrin | 100% | C2 | Anti-fungal |
| Burkholdine | 84% | C3 | Anti-fungal |
| AFC-BC11 | 53% | C3 | Anti-fungal |
| Hydroxyquinolines | 38% | C3 | Signaling molecule/Anti-fungal |
| Cepacin A ^a | 34% | C2 | Anti-oomycetal/Anti-Gram-positive |
| Bactobolins | 27% | C3 | Anti-Gram-negative/Anti-Gram-positive |
| Phenazine | 20% | C2 | Anti-fungal |
| Enacyloxin IIa | 9% | C3 | Anti-Gram-negative |

Footnotes:

^a Cepacin A biosynthetic gene cluster was identified during this study.

Supplementary Table 5. Antimicrobial susceptibility organisms: host and disease phenotypes¹⁻⁴ and incubation temperatures.

| Organism | Source/ID Number | Incubation Temperature (°C) | Host Range | Disease |
|---|------------------|-----------------------------|-----------------------------|--|
| Pathogenic bacteria and fungi | | | | |
| <i>Rhizobium radiobacter</i> | LMG 187 | 30 | Broad | Crown gall disease |
| <i>Pseudomonas savastanoi</i> pv. <i>phaseolicola</i> | LMG 2245 | 30 | Common bean | Bean halo blight |
| <i>Dickeya solani</i> | LMG 25993 | 30 | European potato | Potato tuber rot |
| <i>Pectobacterium carotovorum</i> | LMG 2464 | 30 | Several crop species | Soft rot disease |
| <i>Pseudomonas syringae</i> pv. <i>tomato</i> | LMG 5093 | 30 | Tomato | Bacterial speck |
| <i>Xanthomonas campestris</i> pv. <i>campestris</i> | 8004 | 30 | Cultivated Brassicaceae | Black rot |
| <i>Pseudomonas syringae</i> pv. <i>syringae</i> | LMG 187 | 30 | Common bean | Bacterial brown spot |
| <i>Burkholderia multivorans</i> | ATCC 17616 | 30 | Human (immunocompromised) | Opportunistic (e.g. CF lung infection) |
| <i>Staphylococcus aureus</i> | NCTC 12981 | 37 | Human | Multiple (cutaneous, systemic etc.) |
| <i>Enterococcus faecalis</i> | ATCC 51299 | 37 | Human | Multiple (uninary, systemic etc.) |
| <i>Candida albicans</i> | SC 5314 | 37 | Human | Candidiasis |
| <i>Fusarium solani</i> var. <i>redolens</i> (Wollenweber) | MUCL 14241 | 22 | Multiple crop species | Root/foot rot, wilt |
| <i>Alternaria alternata</i> (Fries:Fries) von Keissler | MUCL 36 | 22 | Several (pathovar specific) | Black-spot, stem canker |
| | | | | |
| Other reference organisms | | | | |
| <i>Bacillus subtilis</i> | ATCC 23857 | 30 | N/A | N/A |

Supplementary Table 6. Minimum Inhibitory Concentration (MIC) of enacyloxin IIa against plant and animal pathogens.

| Organism | MIC (µg/ml) |
|---|-------------|
| <i>Rhizobium radiobacter</i> | 3.2 |
| <i>Pseudomonas savastanoi</i> pv. <i>phaseolicola</i> | 50.0 |
| <i>Dickeya solani</i> | 12.5 |
| <i>Pectobacterium carotovorum</i> | 6.3 |
| <i>Pseudomonas syringae</i> pv. <i>tomato</i> | 50.0 |
| <i>Xanthamonas campestris</i> pv. <i>campestris</i> | 50.0 |
| <i>Pseudomonas syringae</i> pv. <i>syringae</i> | 6.3 |
| <i>Burkholderia multivorans</i> | 6.3 |

Footnotes:

MICs were measured in a microbroth dilution assay using iso-sensitest broth or TSB broth with doubling-dilutions of enacyloxin IIa between 100 and 0.098 µg/ml. Bacteria were grown for 18-24 hours and the optical density at 600 nm was measured. The MIC was determined by calculating the enacyloxin IIa concentration required to produce an 80% knockdown in optical density compared to the organism grown in the absence of the antibiotic; n = 3 replicates for MIC analysis.

Supplementary Table 7. The rhizocompetence of *B. ambifaria* BCC0191 and derived mutants.

| Replicate and Statistics | Colony forming units per gram of root ^a | | |
|--------------------------|--|---------------------|---------------------|
| | WT | :: <i>ccnJ</i> | $\Delta c3$ |
| Replicate 1 | 3.1x10 ⁷ | 1.0x10 ⁷ | 9.0x10 ⁶ |
| Replicate 2 | 1.7x10 ⁷ | 7.9x10 ⁶ | 1.1x10 ⁷ |
| Replicate 3 | 3.6x10 ⁷ | 2.1x10 ⁸ | 5.4x10 ⁶ |
| Mean^b | 2.8x10 ⁷ | 7.8x10 ⁷ | 8.5x10 ⁶ |

Footnote:

^a The total viable counts of each replicate ($n = 3$ plants per condition) from BCC0191 wild-type and mutant derivatives cultured from 14 day colonised root segments and adjusted to 1 g fresh weight of root is shown. No *B. ambifaria* growth was detected on the control root segments.

^b Significant difference ($P = 0.027$, $df = 4$, $t = -4.40$, 95% confidence interval), between WT and $\Delta c3$ as determined by two-sided (unpaired) t-test assuming normally distributed data (Shapiro-Wilk test) and equal variances (Bartlett test). Failing these assumptions, the non-parametric Wilcoxon rank sum test was performed to determine significance.

Supplementary Table 8. Proteins with similarity to those encoded by the cepacin A biosynthetic gene cluster.

| Gene Name | Length bp/aa | Similar Proteins (excluding <i>B. ambifaria</i>) | % aa Sequence Identity | Proposed Function |
|-------------|--------------|--|------------------------|---|
| <i>ccnA</i> | 714/237 | LuxR family transcriptional regulator [Burkholderia vietnamiensis] LuxR family transcriptional regulator [Burkholderia contaminans] LuxR family transcriptional regulator [Burkholderia sp. USM B20] | 82% 83% 82% | LuxR family transcriptional regulator |
| <i>ccnB</i> | 384/127 | Hypothetical Protein [Burkholderia sp. LA-2-3-30-S1-D2] Hypothetical Protein [Burkholderia sp. RF2-non_BP3] Hypothetical Protein [Burkholderia sp. RF4-BP95] | 90% 87% 88% | Hypothetical |
| <i>ccnC</i> | 654/217 | GNAT family N-acetyltransferase [Burkholderia sp. LA-2-3-30-S1-D2] GNAT family N-acetyltransferase [Burkholderia sp. RF4-BP95] GNAT family N-acetyltransferase [Burkholderia contaminans] | 88% 87% 87% | LuxI homoserine lactone synthase |
| <i>ccnD</i> | 1293/430 | beta-ketoacyl-ACP synthase II [Burkholderia sp. LA-2-3-30-S1-D2] beta-ketoacyl-ACP synthase II [Burkholderia sp. RF4-BP95] beta-ketoacyl-ACP synthase II [Burkholderia sp. RF2-non_BP3] | 95% 94% 94% | Beta-ketoacyl synthase |
| <i>ccnE</i> | 1386/461 | NAD(P)/FAD-dependent oxidoreductase [Burkholderia contaminans] NAD(P)/FAD-dependent oxidoreductase [Burkholderia vietnamiensis] NAD(P)/FAD-dependent oxidoreductase [Burkholderia vietnamiensis] | 88% 85% 84% | Flavin-dependent monooxygenase |
| <i>ccnF</i> | 1083/360 | fatty acid desaturase [Burkholderia sp. LA-2-3-30-S1-D2] fatty acid desaturase [Burkholderia sp. RF4-BP95] fatty acid desaturase [Burkholderia vietnamiensis] | 92% 92% 89% | Fatty acid desaturase (alkane hydroxylase) |
| <i>ccnG</i> | 1005/334 | aromatic ring-hydroxylating dioxygenase subunit alpha [Burkholderia contaminans] aromatic ring-hydroxylating dioxygenase subunit alpha [Burkholderia sp. LA-2-3-30-S1-D2] Rieske (2Fe-2S) domain protein [Burkholderia vietnamiensis G4] | 95% 94% 94% | Rieske non-heme iron-dependent oxygenase |
| <i>ccnH</i> | 876/291 | hypothetical protein [Burkholderia contaminans] hypothetical protein [Burkholderia sp. LA-2-3-30-S1-D2] hypothetical protein [Burkholderia sp. RF2-non_BP3] | 86% 82% 81% | Hypothetical |
| <i>ccnI</i> | 1419/472 | MFS transporter [Burkholderia sp. LA-2-3-30-S1-D2] MFS transporter [Burkholderia sp. RF4-BP95] MFS transporter [Burkholderia sp. RF2-non_BP3] | 90% 90% 90% | MFS transporter |
| <i>ccnJ</i> | 1797/598 | AMP-dependent synthetase [Burkholderia contaminans] AMP-dependent synthetase [Burkholderia sp. RF4-BP95] AMP-dependent synthetase [Burkholderia vietnamiensis] | 88% 86% 85% | Fatty acyl-AMP ligase |
| <i>ccnK</i> | 960/319 | acyl-CoA desaturase [Burkholderia contaminans] acyl-CoA desaturase [Burkholderia sp. RF4-BP95] acyl-CoA desaturase [Burkholderia sp. LA-2-3-30-S1-D2] | 92% 93% 92% | Fatty acid desaturase |
| <i>ccnL</i> | 984/327 | acyl-CoA desaturase [Burkholderia contaminans] acyl-CoA desaturase [Burkholderia sp. RF4-BP95] acyl-CoA desaturase [Burkholderia sp. RF2-non_BP3] | 96% 95% 94% | Fatty acid desaturase |
| <i>ccnM</i> | 321/106 | polyketide synthase [Burkholderia sp. RF2-non_BP3] polyketide synthase [Burkholderia sp. LA-2-3-30-S1-D2] polyketide synthase [Burkholderia vietnamiensis] | 96% 95% 95% | Acyl carrier protein |
| <i>ccnN</i> | 1098/365 | fatty acid desaturase [Burkholderia contaminans] fatty acid desaturase [Burkholderia stagnalis] fatty acid desaturase [Burkholderia stagnalis] | 94% 92% 91% | Fatty acid desaturase |
| <i>ccnO</i> | 924/307 | delta-12-desaturase [Burkholderia sp. LA-2-3-30-S1-D2] delta-12-desaturase [Burkholderia vietnamiensis] delta-12-desaturase [Burkholderia contaminans] | 87% 86% 87% | Thioesterase |
| <i>ccnP</i> | 186/61 | rubredoxin [Burkholderia vietnamiensis] rubredoxin [Burkholderia contaminans] rubredoxin [Burkholderia stagnalis] | 95% 93% 92% | Rubredoxin |

Supplementary Table 9. Primers used during this study.

| Primers^a | Final Product Size | Primer Use | Source |
|--|---------------------------|--|---|
| Fwd: 5'- GCG <u>TCT AGA</u> GAC GTG ATC ATT GCC GGA AA -3' Rev: 5'- GCG <u>GAA TTC</u> TTG CCC GAT ACA TAG AGC GT -3' | 707 | Amplify product from fatty AMP ligase-encoding gene, <i>ccnJ</i> | This study |
| Fwd: 5'- TTA YTT TTG YGC CGC TAC MG -3' Rev: 5'- CCM GAG CAG CTY TAT ACG AT -3' | 582 | Screen for presence of c3 replicon in <i>B. ambifaria</i> BCC0191Δc3 (degenerate for <i>B. ambifaria</i>) | This study |
| Fwd: 5'- AAG AAA TCT GCT GCC GCT TG -3' Rev: 5'- CAC TTC GCT GTA CCT CAA GC -3' | 608 | Screen for presence of pMinic3 in <i>B. ambifaria</i> BCC0191Δc3 | This study |
| 5'- AGC GGG CCA A -3' | Variable | Random amplified polymorphic DNA genotyping | Mahenthiralingam <i>et al.</i> ⁷ |

Footnotes:

^a Restriction sites in primer sequences are underlined.

Supplementary Notes

Genome sequencing quality control and assembly. Illumina reads were trimmed using the wrapper script Trim Galore v0.4.2²⁰, which utilises Cutadapt v1.12²¹, and their quality assessed using FastQC v0.10.1²². FLaSH v1.2.11²³ was used to merge overlapping short read pairs to improve contiguity of assembled genomes. The resulting overlapped and paired-end reads output from FLASH were assembled into contigs using the assembler SPAdes v3.9.1²⁴. Misassembled contigs were identified and corrected with Pilon v1.21²⁵. Contig sequences representing contaminating DNA were identified with Kraken v0.10.5-beta²⁶ using the Minikraken database and removed prior to genomic analyses. Genome sequence quality assessment and statistics were calculated using QUAST v4.4²⁷.

***B. ambifaria* genomics and *in silico* definition of specialized metabolite biosynthetic gene clusters.** AntiSMASH analysis²⁸ and BLAST²⁹ searches for known specialized metabolite biosynthetic gene clusters (BGCs) detected a total of 1,272 BGCs across 64 *B. ambifaria* strains, defining a mean of 20 pathways per genome. Eighteen classes of metabolites were identified as well as one distinct BGC that was not recognised as encoding a previously reported metabolite class by antiSMASH, but was recognised as having the potential to direct the production of a specialized metabolite (there were 20 examples of this BGC in the genome set; see Figure 2). A combination of Kmer-matching and gene topology comparisons enabled de-replication of the 1,272 BGCs to 38 distinct putative BGCs (Figure 2; Supplementary Table 3). Of the 38 distinct BGCs, three singleton pathways were detected in contigs that had no significant homology to the reference sequences, and could not be incorporated into the assembled replicons c1, c2 and c3 (Figure 2; Supplementary Table 3).

The specialized metabolite encoding capacity of the replicons relative to their size (density) varied significantly (Supplementary Figure 1). The largest replicon (c1) possessed the lowest specialized metabolite BGC density, whereas the smallest replicon (c3) possessed the largest density. The metabolite BGC density of the replicons was not static across the *B. ambifaria* dataset, with replicon c3 varying in BGC density from less than 10% to above 30% (mean 19.4%; Supplementary Figure 1). Replicons c1 and c2 displayed a lower mean BGC density and density variance compared to c3. *B. ambifaria* strains encoded 3-6 BGCs on replicon c1, with eight distinct BGCs identified on the replicon. Replicon c2 encoded 8-11 BGCs with 17 distinct BGCs; and c3 encoded 4-9 BGCs with eleven distinct BGCs detected.

Of the 38 distinct BGCs, three singleton pathways were detected in contigs that had no significant homology to the reference sequences, and could not be incorporated into the assembled replicons c1, c2 and c3 (Figure 2; Supplementary Table 3). One of BGC encoded a hybrid non-ribosomal peptide synthetase *trans*-acyltransferase polyketide synthase (NRPS-*trans*-AT PKS), and showed 92% sequence similarity to the malleilactone biosynthetic gene cluster^{30,31}. The two remaining BGCs with unknown genomic locations were an uncharacterised NRPS in strain MEX-5, and a LuxRI system in strain IOP40-10. The most frequently detected specialized metabolite classes were terpenes (5/38) and NRPS (4/38). Eleven of the distinct BGCs were encoded by all 64 *B. ambifaria* strains, and included four terpene synthase BGCs and two LuxRI systems (Figure 2). Eight clusters were detected in less than 5% of the *B. ambifaria* strains examined (<3 strains), three of which were type 1 modular PKS gene clusters (Figure 2).

Analysis of QS-regulated BGCs in *B. ambifaria*. The availability of the extensive *B. ambifaria* genome and BGC datasets enabled interrogation of QS regulatory genes with a focus on LuxR-encoding genes as the key BGC regulators. We detected 356 *luxR* homologues across the 64 *B. ambifaria* strains, representing 14 distinct protein phylogenetic clades (Figure 3). These clades included the following *B. ambifaria* LuxRI quorum sensing (QS) systems: the *bafRI* system³² (63 of 64 strains), the *cepR2I2*

system³³ (61 of 64 strains), one functionally uncharacterised system (22 of 64 strains), and a QS system present only in *B. ambifaria* IOP40-10. Six LuxR clades were associated with BGCs encoding the following compounds or compound classes (Figure 3): ectoine, lantipeptide, butyrolactone, enacyloxin IIa, bactobolins and a putative BGC that was identified as directing cepacin biosynthesis (see Figure 4). The remaining LuxR clades flanked membrane transporter genes, a type 3 secretion system and four clades were associated with genes of unknown collective functions (Figure 3).

Phenotypic analysis of *B. ambifaria* BCC0191, cepacin and third replicon mutants. Antimicrobial activity against the panel of plant and animal pathogenic bacteria and fungi, as well as further reference strains, was examined. The loss of cepacin A production in BCC0191::*ccnJ* resulted in loss of anti-Gram-positive activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis* (Supplementary Figure 6), in addition to the loss of *Pythium* inhibition and the weak activity against certain Gram-negative bacteria (Figure 4b; Supplementary Figure 4). Deletion of the third replicon resulted in loss of antagonism against the fungal species *Candida albicans*, *Fusarium solani* and *Alternaria alternata*, but enhanced anti-Gram positive activity which correlated with a 2-fold increase in cepacin production seen in the BCC0191Δc3 mutant (Supplementary Figure 6). The double mutant, BCC0191::*ccnJ*Δc3, lost all the antimicrobial phenotypes observed for the wild-type *B. ambifaria* BCC0191 (Supplementary Figure 6). The rhizocompetence of *B. ambifaria* BCC0191 WT, BCC0191::*ccnJ* and BCC0191Δc3 mutants was also evaluated to assess whether their effect on biological control of *Pythium* (Figure 5) was a consequence of reduced root colonisation (see Supplementary Methods). After 14 days of growth in the pea biological control model system (not containing *P. ultimum*), the wild-type and BCC0191::*ccnJ* colonised the rhizosphere at equivalent levels ($P = 0.7$; Supplementary Table 7). The BCC0191Δc3 mutant colonised the pea rhizosphere at a rate significantly lower ($P = 0.027$, $df = 4$, $t = -4.40$), but within 0.5 log of the mean wild-type level of 2.8×10^7 cfu/g root (Supplementary Table 7).

Supplementary Discussion

Pan-genomics and extensive specialized metabolite diversity. Genomic analysis across 64 *B. ambifaria* genomes revealed substantial diversity in gene content and predicted specialized metabolite BGCs; branching into five defined clades. Eleven of the 38 BGCs were encoded by all studied *B. ambifaria* strains, the remaining BGCs constitute the accessory specialized metabolite potential of *B. ambifaria* (see Figure 2). The large core and accessory metabolite potential in *B. ambifaria* compliments previous studies of the wider *Burkholderia* genus³⁴. Similar in-depth, species-focussed BGC distribution analyses were conducted in three *Salinospora* species with previously unknown metabolite potential, but these were restricted to NRPS, PKS and fatty acid synthase pathways³⁵. A more limited analysis (6 strains) was also conducted with *Streptomyces albus* genomes³⁶. Pan-genomic analysis of nine strains has been performed on the biocontrol species *Pseudomonas putida*³⁷, but a detailed correlation to their specialized metabolite biosynthetic capacity and protective properties, such as that reported in this study, was lacking.

Multifaceted analysis of previously characterised biocontrol strains. We analysed the *in vitro* antimicrobial activity, specialized metabolite BGCs, and metabolite profiles of ten strains spanning the *B. ambifaria* core-gene phylogeny, including seven of the eight previously characterised biological control strains (Supplementary Table 1): AMMD (BCC0207), BC-F (BCC0203), J82 (BCC0191), M54 (BCC0316), Ral-3 (BCC0192), ATCC 53267 (BCC0284) and ATCC 53266 (BCC0338). Most of these biocontrol strains were initially characterised by agricultural and biotechnology companies as potent agents capable of suppressing multiple plant pathogens. Variation was observed in the antimicrobial BGC content of the ten strains. The presence of multiple BGCs encoding broad spectrum antimicrobials suggests a “built-in” redundancy to their biocontrol ability to suppress plant pathogens. Despite encoding antimicrobial BGCs,

there were several examples of non-producing BGCs under the experimental conditions used. The absence of these metabolites in encoding strains may be due to multiple factors such as the presence of BGC or regulatory mutations within these strains, the need for specific growth conditions to prime biosynthesis, or that BGC expression is activated by complex interactions such as contact with a competing organism or inter-kingdom signalling from the host plant.

Effect of third replicon deletion on the pathogenicity of *B. ambifaria* BCC0191. For *B. ambifaria* strains such as AMMD, deletion of the third genomic replicon rendered the derived mutant, AMMD Δ c3, attenuated in multiple virulence models and resulted in loss of anti-fungal activity³⁸, but retained the ability to colonise the rhizosphere³⁹. Our *B. ambifaria* BCC0191 Δ c3 mutant exhibited a non-significant reduction in biocontrol efficacy against *P. ultimum* (10^5 : $P = 0.22$, $t = -1.44$, $df = 4$; 10^6 : $P = 0.22$, $t = -1.46$, $df = 4$; 10^7 : $P = 0.16$, $t = -1.73$, $df = 4$; Figure 5b), but exhibited a significantly reduced rhizocompetence, with the number of viable bacteria per g of root an order of magnitude below that of the wild-type ($P = 0.027$, $t = -3.40$, $df = 4$; Supplementary Table 7). In contrast to AMMD Δ c3³⁸, *B. ambifaria* BCC0191 Δ c3 retained virulence in a *Galleria* wax-moth larvae virulence assay (Supplementary Figure 7a), suggesting in strain BCC0191 *Galleria* virulence is mediated by factors encoded on the first and second replicons. However, corroborating the reduced pathogenicity observed for c3 replicon mutants in vertebrate models³⁸, *B. ambifaria* BCC0191 Δ c3 was not able to persist within the lungs of infected mice (Supplementary Figure 7c). At the equivalent infective dose (2×10^6 bacteria), highly virulent respiratory pathogens such as *Pseudomonas aeruginosa* persist in the same murine infection model at log-fold higher densities in both nasopharynx and lung^{40,41}. In addition, no *B. ambifaria* was detected within the spleens of infected mice suggesting that the capability of this species for invasive infection, observed during fatal “cepacia syndrome” CF infection⁴², is low.

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